with no reference to whether the compound has a *cis* or *trans* configuration.[9] Staudinger and Binkert[10] reported the preparation of stilbenediol dibenzoate, m.p. 159°, along with a small quantity of a material melting at 185–187° from the reaction of benzoyl chloride with the potassium salt of stilbenediol. The material melting at the higher temperature was said to be "probably the α-compound". More recently Ried and Keil[11] referred to the higher melting compound as *trans*-stilbenediol dibenzoate. The compound was formed in low yield (1%) from the reaction of the benzoin–piperidine Mannich base with benzoyl chloride in pyridine. Blake, Coates, and Tate[12] reported the compound melting at 159° as *cis*-stilbenediol dibenzoate.

We have found that both isomers of α,α′-stilbenediol dibenzoate are conveniently prepared by first reducing benzil with potassium metal in refluxing benzene followed by the addition of benzoyl chloride to the reaction mixture. Presumably the potassium salt of stilbenediol is formed prior to the addition of benzoyl chloride.[10] Separation of the two isomers is easily accomplished by taking advantage of the fact that the *cis* isomer is considerably more soluble than the *trans* isomer in benzene.

Experimental[13]

Starting Materials.—Sodium cyanide was commercial material of the highest available purity and was dried for several hours at 100° under vacuum prior to use. Benzil, m.p. 94–95°, was obtained commercially and was used without further purification. Dimethyl sulfoxide[14] was dried with Molecular Sieves and in some cases distilled from calcium hydride (1 mm.).[15] Other materials were of reagent grade and were used as obtained.

Benzil and Sodium Cyanide in Dimethyl Sulfoxide.—Sodium cyanide, 0.49 g. (0.01 mole), was heated with stirring in 80 ml. of dimethyl sulfoxide to 70° under a nitrogen atmosphere. After most of the solute had dissolved, the solution was allowed to cool slowly to room temperature at which time 2.10 g. (0.01 mole) of benzil was added in one portion. The solution instantly became dark brown in color and after a reaction time of 1 min. was poured into cold water. The resulting aqueous suspension was acidified and extracted with ether. The ether solution was washed with water, extracted with sodium bicarbonate solution, washed again with water, dried, and evaporated. The residue, on trituration with ethanol, afforded 1.65 g. (78% yield) of *trans*-α,α′-stilbenediol dibenzoate (II), m.p. 189° (from benzene), lit.[11] m.p. 188.5–189°. On admixture with an authentic sample no depression in melting point was observed.

Anal. Calcd. for $C_{28}H_{20}O_4$: C, 79.89; H, 4.79. Found: C, 79.55; H, 5.15.

The sodium bicarbonate layer was acidified and extracted with ether from which was obtained 0.10 g. of benzoic acid, m.p. 121–122° (from water).

Preparation of *cis*- and *trans*-α,α′-Stilbenediol Dibenzoate.—Potassium metal, 0.78 g. (0.02 g.-atom), was placed in a flask containing 50 ml. of anhydrous benzene. The flask was heated while being stirred in an atmosphere of nitrogen, to the boiling point of the solvent. Benzil, 2.10 g. (0.01 mole), in 25 ml. of benzene was added slowly. When addition was complete the brown solution was stirred for 30 min. followed by the addition of 2.3 ml. (0.02 mole) of benzoyl chloride in 15 ml. of benzene. After stirring for an additional 15 min. the hot reaction mixture was filtered and the inorganic residue was extracted twice with hot benzene. The combined filtrates, after being concentrated to 20 ml. and allowed to cool, afforded 1.53 g. (36% yield) of *trans*-α,α′-stilbenediol dibenzoate (II), m.p. 189° (from benzene), lit.[11] m.p. 188.5–189°.

The filtrate remaining after isolation of the *trans* isomer was evaporated and the residue was triturated with aqueous acetone yielding 1.60 g. (38% yield) of *cis*-α,α′-stilbenediol dibenzoate, m.p. 158–159° (from 95% ethanol), lit.[10] m.p. 159°.

(9) M. S. Kharasch, W. Nudenburg, and S. Archer, *J. Am. Chem. Soc.*, **65**, 495 (1943); M. Gomberg and W. E. Bachmann, *ibid.*, **49**, 2584 (1927); G. Scheuing and A. Heusle, *Ann.*, **440**, 72 (1924); H. Klinger and L. Schmitz, *Ber.*, **24**, 1277 (1891), and references cited therein.

(10) H. Staudinger and A. Binkert, *Helv. Chim. Acta*, **5**, 703 (1922).

(11) W. Ried and G. Keil, *Ann.*, **616**, 96 (1958).

(12) D. Blake, G. E. Coates, and J. M. Tate, *J. Chem. Soc.*, 618 (1961).

(13) Melting points are uncorrected. Elemental analysis was carried out by Swarzkopff Microanalytical Laboratories, Woodside 77, N. Y.

(14) We gratefully acknowledge a free sample of dimethyl sulfoxide from Crown Zellerbach Corp., Camas, Wash.

(15) E. J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, **84**, 866 (1962).

A New Microbiological Steroid Reaction

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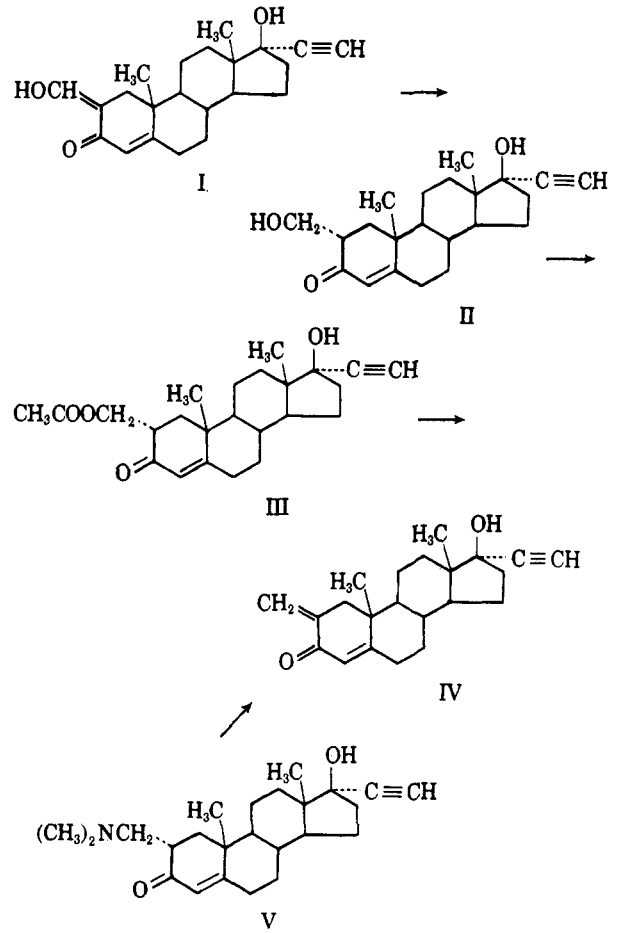
During an investigation[1] of the action of various microorganisms on 2-hydroxymethylene-3-keto steroids,[2] it was observed that when these substrates were submitted to the enzymatic action of *Rhizopus stolonifer* (also known as *Rhizopus nigricans*) an unusual reduction of the β-hydroxy-α,β-unsaturated ketone to a β-hydroxy ketone occurred in preference to the more characteristic reactions of this microorganism (*i.e.*, hydroxylation). This Note reports the microbial reduction of 17α-ethinyl-17-hydroxy-2-hydroxymethyleneandrost-4-en-3-one (I) and the pertinent chemical evidence in support of the assignment of structure II.



(1) M. Riano, *et al.*, to be published.

(2) The preparation of these compounds is reported by A. J. Manson, F. W. Stonner, H. C. Neumann, R. G. Christiansen, R. L. Clarke, J. H. Ackerman, D. F. Page, J. W. Dean, D. K. Phillips, G. O. Potts, A. Arnold, A. L. Beyler, and R. O. Clinton, *J. Med. Chem.*, **6**, 1 (1963), and references cited therein.

17α-Ethinyl-17-hydroxy-2-hydroxymethyleneandrost-4-en-3-one (I) was incubated with *R. stolonifer* ATCC 12939(−) in a medium containing commercial dextrose, Edamine,[3] and corn steep liquor for 38 hr. at 27°. The major product of this reaction, which after purification was obtained in a 10% yield, was assigned structure II on the basis of its elemental analysis and its infrared, n.m.r. and ultraviolet spectra (see Experimental).

This structure was rigorously proved by the following chemical evidence. Acetylation of II with acetic anhydride in pyridine yielded the monoacetate III, which on treatment with sodium bicarbonate in aqueous methanol at room temperature afforded 17α-ethinyl-17-hydroxy-2-methyleneandrost-4-en-3-one (IV). The structure of IV was confirmed by the comparison of the infrared and n.m.r. spectra with those of an authentic sample prepared from 17α-ethinyl-17-hydroxyandrost-4-en-3-one *via* the Mannich base V by the procedure of Carrington and co-workers.[4] Therefore the microbial reduction product must be 17α-ethinyl-17-hydroxy-2-hydroxymethylandrost-4-en-3-one (II).

The configuration of the hydroxymethyl group of II is assumed to be equatorial (*i.e.*, the more stable isomer) due to conditions of its isolation. This assumption is supported by the observation that the infrared spectrum of II shows a ketone maximum at 6.05 μ ($CHCl_3$), an unusually high wave length suggestive of a hydroxy–carbonyl interaction[5] which could only occur if the hydroxymethyl group is in the equatorial configuration. Furthermore, intramolecular hydrogen bonding is indicated by the fact that the relative intensities of the absorption bands of the two hydroxyl groups at 2.76 and 2.83 μ (CCl_4) are not altered by dilution of the solution (*c* 0.004, 0.002, and 0.0002 *M*). Consistent with this line of thought is the normal absorption of the Δ⁴-3-ketone of the monoacetate III at 5.98 μ ($CHCl_3$).

Prior to this Note th eonly reported microbiological reduction of a β-hydroxy-α,β-unsaturated ketone or its tautomer was the reduction of β-ketobutyraldehyde to *d*-1,3-butanediol by yeast.[6] Furthermore, it is noteworthy that *R. stolonifer* is generally associated with oxidative rather than reductive types of microbiological reactions.[7]

Experimental[8]

17α-Ethinyl-17-hydroxy-2α-hydroxymethylandrost-4-en-3-one (II).—A nutrient solution, prepared from 500 g. of commercial dextrose (Cerelose), 200 g. of Edamine,[3] 50 ml. of corn steep liquor, and tap water (*q.s.* 10 l.) was placed in a 14-l. fermentation tank and the solution was sterilized for 45 min. at 15-lb. pressure. The cooled solution was inoculated with a 72-hr. vegetative growth of *R. stolonifer* ATCC 12939(−). (This inoculum was prepared by incubating an aerated 10% suspension of the organism in 1 l. of the above sterile nutrient solution contained in ten 500-ml. Erlenmeyer flasks on a shaker rotating at 210 r.p.m. at 26°.) The culture was allowed to grow for 21 hr. at 27° with an air supply of 3 l./min. and while agitated at 400 r.p.m. After this period of time, a solution of 4 g. of 17α-ethinyl-17-hydroxy-2-hydroxymethyleneandrost-4-en-3-one (I)[2] in 10 ml. of dimethylformamide was added to the culture under aseptic conditions and fermentation was continued under identical conditions for another 38 hr. The fermentation broth was then adjusted to pH 2 by the addition of 6 *N* hydrochloric acid and extracted twice with 20-l. portions of methylene dichloride. The combined extracts were concentrated on a steam bath under reduced pressure to a volume of *ca.* 2 l., washed with 2% sodium bicarbonate solution and water, dried (Na_2SO_4), filtered, and concentrated to dryness to afford 1.55 g. of a residue. The crude product was subjected to chromatography on 50 g. of silica gel. Elution with 5–60% ethyl acetate in ether gave 0.82 g. of colorless solid, which was recrystallized from ethyl acetate. The pure 17α-ethinyl-17-hydroxy-2α-hydroxymethylandrost-4-en-3-one (0.41 g.) formed colorless needles: m.p. 164–165°; $[\alpha]_D$ +38.8°; λ_{max} 242 mμ (ε 14,400); λ_{max} 2.94 (OH), 4.74 (C≡C), 6.06 and 6.18 μ (Δ⁴-3-ketone); $\lambda_{max}^{CCl_4}$ 2.76 (C-17 OH) and 2.83 (bonded OH); $\lambda_{max}^{CHCl_3}$ 6.05 μ (C=O); δ_{max} (10%, $CDCl_3$), 1.28 (C-18 CH_3), 1.80 (C-19 CH_3), 3.12 (≡CH), 4.30 ($-CH_2O-$), and 6.30 p.p.m. (—C(=)CH=).

Anal. Calcd. for $C_{22}H_{30}O_3$: C, 77.16; H, 8.83. Found: C, 77.17; H, 8.83.

In a control experiment, in which only the microorganism was omitted, no amount of II could be demonstrated by careful thin layer chromatography.

2α-Acetoxymethyl-17α-ethinyl-17-hydroxyandrost-4-en-3-one (III).—17α-Ethinyl-17-hydroxy-2α-hydroxymethylandrost-4-en-3-one (II, 174 mg.) was dissolved in a mixture of 0.5 ml. of pyridine and 1 ml. of acetic anhydride. After standing for 4 hr. at room temperature the mixture was quenched in ice–water and the precipitated material was collected, air dried, and recrystallized from ether–hexane. The monoacetate (78 mg.) had a m.p. 86–89°; $[\alpha]_D$ +20.2; λ_{max} 241 mμ (ε 12,600); λ_{max} 2.88 (OH), 3.04 (≡CH), 4.75 (C≡C), 5.74 (C=O of acetate), and 5.98 and 6.19 μ (Δ⁴-3-ketone); $\lambda_{max}^{CHCl_3}$ 5.75 (C=O of acetate) and 5.98 μ (Δ⁴-3-ketone).

Anal. Calcd. for $C_{24}H_{32}O_4$: C, 74.97; H, 8.39. Found: C, 74.67; H, 8.49.

17α-Ethinyl-17-hydroxy-2-methyleneandrost-4-en-3-one (IV). A. From 2α-Acetoxymethyl-17α-ethinyl-17-hydroxyandrost-4-en-3-one (III).—A solution of 0.60 g. of III in 25 ml. of methanol was added to a solution of 1.2 g. of potassium bicarbonate in 100 ml. of 80% aqueous methanol. After 30 hr. at room temperature the mixture was concentrated to a small volume under reduced pressure. The residue was mixed with 50 ml. of water and extracted with ethyl acetate. The extract was dried (Na_2SO_4), filtered, and evaporated to dryness. There remained an oil which was dissolved in a minimum amount of ethyl acetate. On the addition of hexane to this solution, a solid precipitate (0.30 g.) was obtained. Thin layer chromatography (silicon dioxide–ethyl acetate) of this solid revealed two spots of equal intensities as detected by sulfuric acid treatment followed by heat. One of these spots had a R_f value that was identical with that of the R_f value of 17α-ethinyl-17-hydroxy-2α-hydroxymethylandrost-4-en-3-one. The other, less polar spot, was assumed to be the title compound. The crude mixture was subjected to chromatography on 40 g. of Florisil. Elution with 10% ether in benzene gave a series of fractions which was combined (0.05 g.) and recrystallized from ethyl acetate and pentane to afford 17α-ethinyl-17-hydroxy-2-methyleneandrost-4-en-3-one as colorless, rectangular plates: m.p. 184–185°; $[\alpha]_D$ = + 70.0 (C=O, 4%); λ_{max} 260 mμ (ε 13,900); λ_{max} 2.90 (OH), 4.75 (C≡C), and 6.00 and 6.15 μ (Δ⁴-3-ketone); δ_{max} (12% $CDCl_3$), 1.45 (C-18 CH_3), 1.67 (C-19 CH_3), 3.04 (≡CH), 5.80 and 6.50 (=CH_2), and 6.42 p.p.m. (—C(=)CH=).[9]

(3) An enzymatic digest of lactalbumin obtained from Sheffield Farms' New York, N. Y.

(4) T. R. Carrington, A. G. Long, and A. F. Turner, *J. Chem. Soc.*, 1572 (1962). The synthesis of IV by still another method (*i.e.*, aldol condensation of the corresponding 2-ethoxyoxalyl derivative and formaldehyde) was recently reported by D. D. Evans, D. E. Evans, G. S. Lewis, and P. J. Palmer, *ibid.*, 4312 (1963).

(5) (a) R. N. Jones, P. Humphries, F. Herling, and K. Dobriner, *J. Am. Chem. Soc.*, **74**, 2820 (1952); (b) A. C. Huitric and W. D. Kumler, *ibid.*, **78**, 1147 (1956).

(6) S. Grzycki, *Biochem. Z.*, **265**, 195 (1933).

(7) S. C. Prescott and C. G. Dunn, Industrial Microbiology, 3rd Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1959, Chapter 44.

(8) All melting points are corrected. Except as noted, rotations were measured in chloroform (*c* ~1) solution at 25°, ultraviolet spectra in 95% ethanol (Cary), and infrared spectra in a KBr disk (Perkin-Elmer 21). Nuclear magnetic resonance spectra were determined on a Varian A-60 spectrometer using tetramethylsilane as an external standard and the signal assignments were consistent with the observed intensities and line shapes.

(9) *Cf.* assignment of n.m.r. signals for 2-methylene-17α-methyltestosterone, J. A. Edwards, M. C. Calzada and A. Bowers, *J. Med. Chem.*, **6**, 178 (1963).

Anal. Calcd. for $C_{22}H_{28}O_2$: C, 81.44; H, 8.70. Found: C, 81.66; H, 8.45.

B. From 17α-Ethinyl-17-hydroxyandrost-4-en-3-one *via* Mannich Reaction.—A solution of 14.0 g. of 17α-ethinyl-17-hydroxyandrost-4-en-3-one, 7.0 ml. of 40% aqueous formaldehyde, and 7.0 g. of dimethylamine hydrochloride in 300 ml. of acetic acid was heated in a nitrogen atmosphere at 80° for 1 hr. The reaction mixture was poured into 2 l. of ice-cold water. The resultant precipitate was collected on a filter and dried. This material (1.04 g.) proved to be recovered starting material. The filtrate was made neutral with saturated sodium bicarbonate solution and extracted with methylene dichloride. The organic extract was washed with saturated salt solution, dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure to afford 14 g. of a solid. (Purification of a small sample of this base by recrystallization from methylene dichloride was unsuccessful, probably owing to its ready decomposition to the 2-methylene derivative.) The crude Mannich base was decomposed to the 2-methylene derivative by subjecting it to chromatography on 400 g. of Florisil. Elution with methylene dichloride–ether (1:1) gave 3.69 g. of crude 2-methylene derivative, m.p. 167–174°, which was shown by thin layer chromatography to be partly contaminated with starting material. The crude product was subjected to a second purification by chromatography on 300 g. of Florisil. Elution with benzene–ether (9:1) and two recrystallizations from acetone afforded 1.02 g. of pure 17α-ethinyl-17-hydroxy-2-methyleneandrost-4-en-3-one as colorless plates, m.p. 182–184°, undepressed upon admixture with the sample prepared by method A. The infrared and n.m.r. spectra of this sample were identical with those of the sample prepared by method A.

Acknowledgment.—The authors are indebted to Dr. F. C. Nachod, Miss C. M. Martini, and Mr. M. Priznar for spectral determinations, and to Mr. K. D. Fleischer and staff for analytical services.

The Alkaloids of *Tabernanthe iboga*. IX.[1] The Structures of the Ibogaline Derivatives, Kisantine and Gabonine

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Six years ago we reported[2] the isolation of twelve alkaloids from *Tabernanthe iboga* Baillon and determined the structures of nine of them.[3] Of the remaining three bases, which had been obtained in very small amounts, kimvuline has been shown to be identical with iboxygaine[4] and now kisantine and gabonine are recognized to be oxidation products of ibogaline (I),[5] which contain the oxindole (II) and the *o*-acetamidoacetophenone (III) chromophores, respectively.

Kisantine (II) [λ_{max} mμ (ε), 213 (31,700), 268–270 (6270) and sh 296 (4710); $\nu_{C=O}^{Nujol}$ 1670 cm.$^{-1}$] has spectral properties in agreement for a 5,6-dimethoxyoxindole chromophore; *cf.* carapanaubine[6] or 5,6-dimethoxyoxindole itself [λ_{max} mμ (ε), 264 (7440) and sh 299 (3600); $\nu_{C=O}^{Nujol}$ 1710 and 1680 cm.$^{-1}$].[7] This conclusion is borne out in the p.m.r. spectrum[8] which showed the presence of a strong singlet peak at 3.9 (2-MeO), two singlet peaks at 7.1 and 6.56 (two uncoupled aromatic protons), a singlet at 9.1 (NH), and the characteristic set of peaks at *ca.* 1 p.p.m. for the CH_3 of the ethyl moiety.

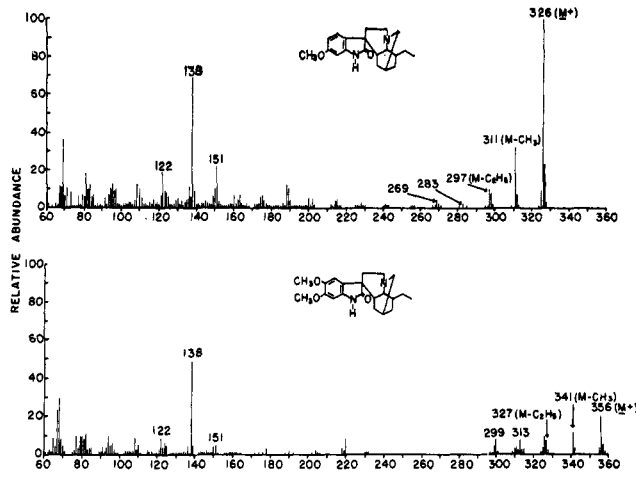
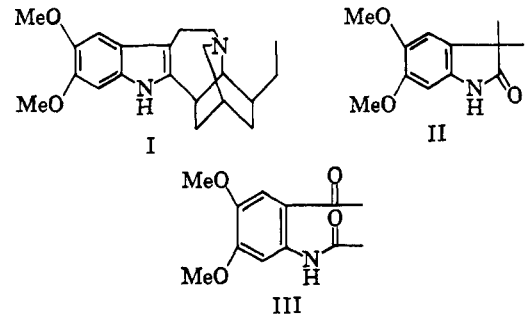


Figure 1.—Tabernanthine oxindole (above) and kisantine (II) (below).



Confirmation of these conclusions were obtained from a comparison of the mass spectra[9] of tabernanthine oxindole[3] and kisantine (Figure 1) which differ only in respect to those peaks which contain the extra methoxyl group.

Gabonine has an ultraviolet absorption spectrum [λ_{max} mμ (ε, calcd. for monomer), 250 (24,020), 283 (6620), and 348–350 (5530)] similar to that recorded for N-ethoxalyl-*o*-aminoacetophenone[10] and almost superimposable upon that of 2-acetamido-4,5-dimethoxybenzaldehyde [λ_{max} mμ (ε), 251 (35,200), 290 (9640), and 344 (7990)]. The p.m.r. spectrum[7] showed the expected singlet peaks at 10.15 (NH), 8.1 and 7.0 (the two aromatic protons), 3.95 and 3.90 (the two MeO), and the set of peaks *ca.* 1 p.p.m. (CH_3 of an ethyl group).

(1) Part VIII. M. F. Bartlett, D. F. Dickel, R. C. Maxfield, L. E. Paszek, and A. F. Smith, *J. Am. Chem. Soc.*, **81**, 1932 (1959).

(2) D. F. Dickel, C. L. Holden, R. C. Maxfield, L. E. Paszek, and W. I. Taylor, *ibid.*, **80**, 123 (1958).

(3) M. F. Bartlett, D. F. Dickel, and W. I. Taylor, *ibid.*, **80**, 126 (1958).

(4) R. Goutarel, F. Percheron, and M.-M. Janot, *Compt. rend.*, **246**, 279 (1958). Iboxygaine (sample from M.-M. Janot) gives an undepressed mixture melting point and a superimposable infrared spectrum in Nujol with kimvuline.

(5) N. Neuss, *J. Org. Chem.*, **24**, 2047 (1959).

(6) B. Gilbert, J. A. Brissolese, N. Finch, W. I. Taylor, H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *ibid.*, **85**, 1523 (1963).

(7) These and other simple oxindoles [G. N. Walker, *J. Am. Chem. Soc.*, **77**, 3845 (1955)] show two strong bands in the carbonyl region not shown by their 3,3-disubstituted derivatives, *e.g.*, the oxindole alkaloids; ref. 6 and N. Finch and W. I. Taylor, *ibid.*, **84**, 3871 (1962), and references therein.

(8) The p.m.r. spectra were run in deuteriochloroform on a Varian A-60 using tetramethylsilane as an internal standard. The shifts are reported in parts per million using the tetramethylsilane signal as reference.

(9) The mass spectra were determined for us through the kindness of Dr. C. Djerassi.

(10) R. Goutarel, M.-M. Janot, V. Prelog, and W. I. Taylor, *Helv. Chim. Acta*, **33**, 150 (1950).